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NEWS 7	FEB 27	New STN AnaVist pricing effective March 1, 2006
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NEWS 14	APR 12	Improved structure highlighting in FQHIT and QHIT display in MARPAT
NEWS 15	APR 12	Derwent World Patents Index to be reloaded and enhanced during second quarter; strategies may be affected
NEWS 16	MAY 10	CA/CAplus enhanced with 1900-1906 U.S. patent records
NEWS 17	MAY 11	KOREAPAT updates resume
NEWS 18	MAY 19	Derwent World Patents Index to be reloaded and enhanced
NEWS 19	MAY 30	IPC 8 Rolled-up Core codes added to CA/CAplus and USPATFULL/USPAT2
NEWS 20	MAY 30	The F-Term thesaurus is now available in CA/CAplus
NEWS 21	JUN 02	The first reclassification of IPC codes now complete in INPADOC

NEWS EXPRESS JUNE 16 CURRENT WINDOWS VERSION IS V8.01b, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 23 MAY 2006.

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FILE LAST UPDATED:         13 JUN 2006      <20060613/UP>
MOST RECENT UPDATE WEEK:       200623        <200623/EW>
FILE COVERS 1978 TO DATE

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    SEE
    http://www.stn-international.de/stndatabases/details/ipc-reform.html >>>

>>> FOR CHANGES IN PCTFULL PLEASE SEE HELP CHANGE
    (last updated April 10, 2006) <<<

=> s annexin
          2779 ANNEXIN
           222 ANNEXINS
L1        2848 ANNEXIN
                 (ANNEXIN OR ANNEXINS)

=> s l1/ab or l1/ti or l1/clm
            65 ANNEXIN/AB
            10 ANNEXINS/AB
            69 ANNEXIN/AB
                 ((ANNEXIN OR ANNEXINS)/AB)
            25 ANNEXIN/TI
             6 ANNEXINS/TI
            30 ANNEXIN/TI
                 ((ANNEXIN OR ANNEXINS)/TI)
           287 ANNEXIN/CLM
L2         297 (ANNEXIN/AB) OR (ANNEXIN/TI) OR (ANNEXIN/CLM)

=> s cancer? or neoplas? or tumor?
         78950 CANCER?
         22900 NEOPLAS?
         65926 TUMOR?
L3       98312 CANCER? OR NEOPLAS? OR TUMOR?

=> s l2 and l3
L4         252 L2 AND L3

=> s l4 not py>1998
        758464 PY>1998
L5          13 L4 NOT PY>1998

=> s lung and l5
         76462 LUNG
         17733 LUNGS
         82912 LUNG
                 (LUNG OR LUNGS)
L6           9 LUNG AND L5

=> s immune or humoral or immunization or immunity or immunizing
         61380 IMMUNE
          2267 IMMUNES
         61964 IMMUNE
                 (IMMUNE OR IMMUNES)
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          9707 HUMORAL
         19694 IMMUNIZATION
          4681 IMMUNIZATIONS
         20347 IMMUNIZATION
                 (IMMUNIZATION OR IMMUNIZATIONS)
         21304 IMMUNITY
           105 IMMUNITIES
         21324 IMMUNITY
                 (IMMUNITY OR IMMUNITIES)
         11679 IMMUNIZING
L7       71233 IMMUNE OR HUMORAL OR IMMUNIZATION OR IMMUNITY OR IMMUNIZING

=> s l7 and l6
L8           4 L7 AND L6

=> d ibib 1-4

L8     ANSWER 1 OF 4     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        1998048699 PCTFULL  ED 20020514
TITLE (ENGLISH):         METHOD OF IMAGING CELL DEATH IN VIVO
TITLE (FRENCH):          PROCEDE D'IMAGERIE DE LA MORT CELLULAIRE IN VIVO
INVENTOR(S):             BLANKENBERG, Francis, G.;
                         STRAUSS, H., W.;
                         TAIT, Jonathan, F.;
                         KATSIKIS, Peter, D.
PATENT ASSIGNEE(S):      THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR
                         UNIVERSITY;
                         UNIVERSITY OF WASHINGTON
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER              KIND     DATE
                         ---------------------------------
                         WO 9848699            A1 19981105
DESIGNATED STATES
       W:                AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE
                         ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC
                         LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU
                         SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH GM
                         KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE
                         CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ
                         CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.:       WO 1998-US8769       A  19980428
PRIORITY INFO.:          US 1997-60/045,399      19970430

L8     ANSWER 2 OF 4     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        1998013494 PCTFULL  ED 20020514
TITLE (ENGLISH):         WILMS' TUMOR WTI BINDING PROTEINS
TITLE (FRENCH):          PROTEINE FIXATRICE DU GENE WT1 SUPPRESSEUR DE LA TUMEUR
                         DE WILM
INVENTOR(S):             SHI, Yang
PATENT ASSIGNEE(S):      PRESIDENT AND FELLOWS OF HARVARD COLLEGE;
                         SHI, Yang
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER              KIND     DATE
                         ---------------------------------
                         WO 9813494            A2 19980402
DESIGNATED STATES
       W:                AU CA JP NZ US AT BE CH DE DK ES FI FR GB GR IE IT LU
                         MC NL PT SE
APPLICATION INFO.:       WO 1997-US17382      A  19970926
PRIORITY INFO.:          US 1996-60/028,923      19960927
```

L8 ANSWER 3 OF 4 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1996003655 PCTFULL ED 20020514
TITLE (ENGLISH): METHOD FOR THE DETERMINATION OF THE PRETHROMBOTIC STATE
TITLE (FRENCH): PROCEDE POUR LA DETERMINATION D'UN ETAT PRETHROMBOTIQUE
INVENTOR(S): FREYSSINET, Jean-Marie;
ANTONI, Benedicte;
DONIE, Frederic;
LILL, Helmut
PATENT ASSIGNEE(S): BOEHRINGER MANNHEIM GMBH;
FREYSSINET, Jean-Marie;
ANTONI, Benedicte;
DONIE, Frederic;
LILL, Helmut
LANGUAGE OF PUBL.: German
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9603655	A1	19960208

DESIGNATED STATES
W: JP US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1995-EP2846 A 19950719
PRIORITY INFO.: AT 1994-94111514.9 19940723

L8 ANSWER 4 OF 4 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1995015979 PCTFULL ED 20020514
TITLE (ENGLISH): PRETARGETING METHODS AND COMPOUNDS
TITLE (FRENCH): PROCEDES ET COMPOSES DE PRECIBLAGE
INVENTOR(S): THEODORE, Louis, J.;
MEYER, Damon, L.;
MALLETT, Robert, W.;
KASINA, Sudhakar;
RENO, John, M.;
AXWORTHY, Donald, B.;
GUSTAVSON, Linda, M.
PATENT ASSIGNEE(S): NEORX CORPORATION
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9515979	A1	19950615

DESIGNATED STATES
W: CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1994-US14174 A 19941207
PRIORITY INFO.: US 1993-8/163,188 19931207

=> d ibib abs kwic 2

L8 ANSWER 2 OF 4 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1998013494 PCTFULL ED 20020514
TITLE (ENGLISH): WILMS' TUMOR WTI BINDING PROTEINS
TITLE (FRENCH): PROTEINE FIXATRICE DU GENE WT1 SUPPRESSEUR DE LA TUMEUR DE WILM
INVENTOR(S): SHI, Yang
PATENT ASSIGNEE(S): PRESIDENT AND FELLOWS OF HARVARD COLLEGE;
SHI, Yang
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9813494	A2	19980402

DESIGNATED STATES

W: AU CA JP NZ US AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE

APPLICATION INFO.: WO 1997-US17382 A 19970926

PRIORITY INFO.: US 1996-60/028,923 19960927

ABEN The present invention comprises compositions and methods that relate to diagnostic and therapeutic assays and reagents for detecting and treating disorders involving aberrant assembly of WT1 complexes. Moreover, drug discovery assays are provided for identifying agents which can modulate the binding of one or more of the subject WT1-binding proteins with WT1 or other transcriptional regulatory proteins. Such agents can be useful therapeutically to alter the growth and/or differentiation of a cell, but can also be used in vitro as cell-culture additives for controlling proliferation and/or differentiation of cultured cells and tissue.

ABFR L'invention concerne des compositions et des methodes relatives a des examens diagnostiques et therapeutiques et des reactifs pour la detection et le traitement de troubles impliquant l'assemblage aberrant de complexes de WT1. Elle se rapporte encore a des examens de recherche utilises pour l'identification d'agents pouvant moduler la liaison d'une ou plusieurs proteines fixatrices de WTde l'invention a WT1 ou d'autres proteines de regulation transcriptionnelles. Lesdits agents peuvent etre utiles sur le plan therapeutique pour la modification de la croissance et/ou la differentiation cellulaire, mais egalement in vitro en tant qu'additifs a la culture cellulaire pour la modulation de la proliferation et/ou la differentiation de cellules et de tissu mis en culture.

TIEN WILMS' TUMOR WTI BINDING PROTEINS

DETD WILMS' TUMOR WTI BINDING PROTEINS

Background of the Invention

Wilm's tumor is a pediatric nephroblastoma that is one of the most frequent solid tumors in children, occurring in about one in ten thousand live births. The tumor appears to arise from the embryonal metanephric mesenchyme during differentiative events in the embryonic kidney and is often composed of stromal, epithelia]. . .

Wilm's tumors (WT) are associated with certain congenital defects, in particular, 1 5 sporadic aniridia (a malfon-nation of the iris and surrounding tissue), hemihypertrophy, Beckwith-Wiedemann Syndrome (BWS, a congenital overgrowth syndrome characterized by growth abnormalities and a predisposition to several embryonal neoplasms. including WT), Denys Drash Syndrome (DIDS, which consists of the triad WT, intersex disorders, and nephropathy), and various anomalies of the. . .

Wilm's tumor has been linked to inactivation of the tumor-suppressor gene WTI at the I IpI 3 chromosomal locus (Haber et al. (I 992) Adv Cancer Res 59:41-68).

WTI encodes a developmentally regulated transcription factor of 52-54 kDa. The Wilms
tumor candidate gene WTI is a tumor suppressor gene expressed in the developing
kidney and in the adult urogenital system (reviewed in Haber, D. A. et al. (I. . . et al. (I 996)
Biochimica et Biophysica Acta 1287:1-28). The WTI gene is deleted or mutated in
approximately 10% of sporadic Wilms tumors and in nearly 100% of Denys-Drash
patients (reviewed in Dignam, J. D. et al. (1983) Nucleic Acids Res 11:1475-1489;
Haber, D. A. et al. (I 992) Adv. Cancer. Res. 59:41-68). WTI protein has been shown to
suppress cell growth in both Wilms tumor and non-Wilms' tumor cells (Haber, D. A. et
al. (1993) Science 262:2057-2059; Kudoh, T. et al. (1995) Proc. Natt. Acad Sci. USA
92:4517-4521; Luo, X.-N.. . .

. . .

formed,
the gonadal ridge mesothelia, and the mesothelial lining of the coelomic cavity and the
organs it contains (Sharma et al. (1992) Cancer Research 52:6407-6412; and Jones et al.

. . .

or activating other cellular genes. The expression pattern of WTI observed in a number of species supports its role as a tumor suppressor gene in
kidney, and extends its possible functions to differentiation events in other organs.

. . .

11

(Drummond et al., supra), and EGR-I genes (Madden et al. (1991) Science 253:1550-
1553), supporting the potential of WTI as a tumor suppressor. Classification of WTI as
a tumor suppressor gene is based on detection in tumor specimens of mutations within
genes that inactivate the protein, such as small deletions and point mutations in the zinc
fingers of WTI that abolish DNA binding in a number of Wilm's tumors (Little et al.

. . .

that are defective for
transcriptional repression activity (Haber, D. A. et al. (I 993) Science 262:2057-2059;
Park, S. et al. (I 993) Cancer Res. 53:475 7-4760). Heterozygous WTI mutations are
also associated with the disease. These WTI mutants were shown to function as
dominant negatives. . .

(1992)Nature56:215-221). Inaddition,analysesofWilms' tumorsamplesrevealedthat
the majority of Wilms tumors do not contain p53 mutations (Malkin, D. et al. (1994)
Cancer Res. 54:2077-2079), although p53 defects are associated with anaplastic Wilms'
tumors (Bardeesy, N. et al. (1994) Nature Genetics 7:91-96). To systematically identify
proteins that interact with WTI, and to determine the functional. . .

. . .

the Invention

The present invention relates to the discovery in eukaryotic cells,

particularly
human cells, of novel protein-protein interactions between the Wilms tumor regulatory
protein WTI and certain cellular proteins, referred to hereinafter as WTI-binding
proteins or WTI-BP.

. . .
an immunogen comprising a
Wtl-binding protein, for example a WTI-13POOO-1 peptide in an immunogenic
preparation, the immunogen being capable of eliciting an immune response specific for
said WTI-BP polypeptide; e.g. a humoral response, e.g. an antibody response- e.g. a
cellular response. In preferred embodiments, the immunogen comprises an antigenic
determinant. e.g. a unique determinant,. . .

. . .
of the present invention concerns an immunogen comprising a par-4 polypeptide in an immunogenic preparation, the immunogen being capable of
eliciting an immune response specific for said polypeptide; e.g. a humoral response, e.g.

. . .
a
modulation, e.g., inhibition, of the interaction between WTI and the WTI-binding
protein. In preferred embodiments: the WTI protein is a Wilm's tumor WTI protein.

Immunoprecipitations were performed with a-WT I Or pre-immune rabbit serum
(indicated NRS). The immunoprecipitates were analyzed for the presence of FLAGpar-4
by Western blot with anti-FLAG (cc-FLAG) monoclonal antibody. Molecular.
. .

. . .
WTI and par-4 in the Ml 5 mouse
mesonephric cells. The Ml 5 whole cell extracts were subjected to immunoprecipitation
with either pre-immune rabbit serum (NRS) or affinity-purified anti-par-4 (cc-par-4)
antibodies. The immunoprecipitated proteins were analyzed by Western blotting using
cc-WTI antibodies. The position of. . .

Detailed Description of the Invention
Wilm's Tumor has been linked to the inactivation of the WTI tumor-suppressor
gene at the 1 1 pl 3 chromosomal locus (Haber et al. (1 992) Adv Cancer Res 59:41-68).

. . .
of
transcription initiated from the promoters of, for example, the PDGF A-chain, EGR-I,
and IGF-11 genes is a general paradigm for the tumor suppressor activity of WT-I and
indicates ability of the protein to directly mediate organogenesis through its influence on
the transcription of growth-related. . .

. . .
genes that
are involved in, for example, epithelial differentiation. This invention derives in part
from the discovery that, in addition to the tumor suppressor

protein p53, the Wilms
tumor suppressor protein WTI is also associated with several
other cellular proteins
(hereinafter termed cellular WTI-binding proteins or WTI-binding
proteins or
WTI-BPs), which association is likely to be involved in WTI-mediated
gene
expression and presumably important in the pathogenesis of Wilms
tumor disease states
as well as other proliferative and differentiative disorders. For
example, association of
one or more of the subject WTI-binding proteins. . . control
of entry into the apoptosis pathway, in the maintenance and survival of
differentiated
tissues, as well as in development of Wilms tumor and other
neoplastic abnon-nalities.

. . .
mesoderm, or alternatively, ectoden-nally-derived spinal or
brain tissue: the ability to modulate differentiation of renal tissue in
normal kidneys or in
Wilm's tumors; the ability to modulate differentiation of
urogenital tissue, such as
1 5 normal or neoplastic gonadal tissue, e.g. ovarian tissue,
e.g. gonadal tissue (such as
granulosa and Sertoli cells); the ability to modulate differentiation of
heart. . .

. . .
a WT1-binding protein can be obtained by
isolating total mRNA from a cell, e.g. a mammalian cell, e.g. a human
cell, including
tumor cells. Double stranded cDNAs can then be prepared from
the total mRNA, and
subsequently inserted into a suitable plasmid or bacteriophage. . .

. . .
antisense therapy
have been reviewed, for example, by van der Krol et al. (1988)
Biotechniques 6:959-
976; and Stein et al. (1988) Cancer Res 48:2659
Accordingly, the modified oligomers of the invention are useful in
therapeutic,
diagnostic, and research applications. In therapeutic applications, the.
. .

. . .
it bind are misexpressed. For example, gene therapy can be used to
deliver a gene encoding a WT-binding protein which inhibits
neoplastic transformation,
such as in the generation of Wilm's tumor, by interfering with
the biological function of
WTI.

. . .
for the present invention. The gene therapy constructs of the
present invention are intended for cells, for example, of a Wilm's
tumor, for example for
use of antagonistic forms of WTI-BpCiao-I or WTI-Bppor-4
. The limitation on
infection of dividing cells is beneficial, in that surrounding tissue
comprising
nontransfon-ned cells do not undergo as rapid cell division as target
tumor cells, and is
therefore more refractory to infection with a retroviral vector.

For example, lipofection of Wilms tumor cells can be carried
out using liposomes tagged
with monoclonal antibodies against, for example, the Thy-I antigen, the
cell adhesion

molecule NCAM, carbohydrate antigen 125 (CA125), or any other cell surface antigen
present on the tumor cells.

. . .

delivered in a gene therapy construct to a cell by electroporation using techniques described, for example, by Dev et al. ((1994) Cancer Treat Rev
20:105-115).

. . .

tissue, and also
for non-renal tissue, such as in controlling the development and maintenance of tissue
from the digestive tract, pancreas, spleen, lungs, and other organs which derive from the
1 5 primitive gut, as well as lateral and intermediate mesoden-n-derived structures including
urogenital structures. . .

. . .

is able to bind to itself
In an exemplary embodiment, the present method can be used in the treatment of
hyperplastic and neoplastic disorders, particularly those characterized by mis-expression
of proteins involved in regulatory complexes which include the subject WTI-binding
proteins, e.g., complexes involving WTI. For instance, nephroblastomas (e.g. Wilms
tumors) are marked by abnormal proliferation of renal cells in which alteration in WTI
function is implicated in the pathogenesis of tumor progression. The change in W77
regulatory function is presumbaly due to mechanisms which involve either disruption of
WTI repressor activity, e.g., of. . . proteins and selected by the methods described here are contemplated for a
- 34 -
variety of therapeutic applications including suppression of tumors and metastases and
neurological defects.

Furthermore, WTI is deleted or mutated in only 10 percent of sporadic Wilms
tumors, implicating other proteins which act close to WTI as potential sites for
transforming genetic lesions. Accordingly, each of the subject WTI -binding proteins are
potential alternate loci in the development of Wilms tumor. In those situations, effective
therapeutic intervention may be provided by complementation with agonists of that
dysfunctional WTI -binding protein, as for example,. . .

. . .

the complexes formed by at least one of the
subject WTI -binding proteins and WTI (or other regulatory proteins), may nevertheless
provide anti-neoplastic treatment regimens for tumor therapy.

. . .

other tissue in which events
mediated by one or more of the subject WTI -binding proteins may contribute in some
manner to neoplastic transformation. For instance, analogous to epithelial cells of the
renal tube (which are transformed in Wilms tumor at the metanephronic blastema stage

of development), mesothelial cells, e.g. epithelial cell layers which line serosal surfaces, undergo mesenchymal-epithelial transition during development. Moreover, there are histologically similar characteristics between mesotheliomas., the tumors which arise from mesothelium, and nephroblastomas, including the apparent involvement of WTI (Walker et al. (1994) Cancer Res 54:3101-3106). Consequently, discovery of the interaction between WTI and the subject WTI-binding proteins, as well as the concomitant realization of potential. . .

. . .

mesothelioma cells with an agent that modulates the function of a WTI -binding protein may, as in the case of Wilms tumor therapies, inhibit tumor cell growth by causing differentiation of tumorogenic cells to post-mitotic tissue and/or cell death.

. . .

Moreover, the expression of WTI in other tissue, such as the bladder, testis and other urogenital tissue, as well as in lung, heart and spleen tissue, shows that corresponding modes of intervention may exist for neoplasias and hyperplasias.

. . .

in hematopoietic cells, indicating a potential role for that protein in hematopoietic differentiation and regulation, as well as possible involvement in hematopoietic tumors.

In yet another embodiment, the subject method can be used in the treatment of neoplastic or hyperplastic transformations of the central nervous system. For instance, certain of the WTI-binding proteins are likely to be involved in. . . neuronal differentiation, may involve, for example, disruption of autocrine loops, e.g., TGF-b or PDGF autostimulatory loops, believed to be involved in the neoplastic transformation of several neuronal tumors. Accordingly, the subject method may be of use in the treatment of, for example, malignant gliomas, medulloblastomas, neuroectodermal tumors, and ependymonas.

. . .

subject WTI-binding proteins in controlling the development of stem cells responsible for formation of the kidneys, as well as of the pancreas, lungs and other organs which derive from the primitive gut. For example, therapeutic compositions for modulating the action of one or more. . .

Similarly, therapeutic compositions targeting WTI -BP function may be useful to promote regeneration of lung tissue in the treatment of emphysema and other degenerative conditions of the lung. For example, the subject method may useful in the treatment of degenerative disorders of lung tissue caused by, for instance, toxic injuries, as well as inflammatory and degenerative processes induced by viral infections. Tissue degeneration of the lung which may be treatable by the present

invention includes
degenerative changes affecting the endothelial and epithelial cells, basal membrane, and
mesenchymal and. . .
. . .
nervous system, including traumatic injury, chemical injury, vasal injury and deficits (such as the ischemia resulting from stroke), together with
infectious/inflammatory and tumor-induced injury; (ii) chronic neurodegenerative
diseases of the nervous system, including Parkinson's disease, Huntington's chorea,
amylotrophic lateral sclerosis and the like, as well. . .

The Multiple Antigen Peptide system for peptide-based immunization can also be
utilized to generate an immunogen, wherein a desired portion of a subject WTI-binding
protein is obtained directly from organo-chemical. . .
. . .
in the art. An
immunogenic portion of the subject WTI-binding proteins can be administered in the
presence of adjuvant. The progress of immunization can be monitored by detection of
antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used
with the. . . .

Following immunization, anti-WTI -BP antisera can be obtained and, if desired,
polyclonal anti-WTI-BP antibodies isolated from the serum. To produce monoclonal
antibodies, antibody producing. . . al., (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to
produce human monoclonal antibodies (Cole et al., (1985) Monoclonal Antibodies and
Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened
immunochemically for production of antibodies specifically reactive with a. . .
. . .
and allow the study of the role of a
particular WTI-binding protein in transcriptional regulation generally, or in the etiology
of Wilm's tumors specifically, e.g. by microinjection of
anti-WTI -BP into cells.
. . .
level of WTI-BP/WTI complexes
can be useful in predictive valuations of the onset or progression of, for example,
nephroblastomas such as Wilm's tumors. Likewise, the ability to monitor WTI-BP
levels in the cells of an individual can permit determination of the efficacy of a. . .

Diagnostic assays using anti-WTI-BP antibodies can include, for example, immunoassays designed to aid in early diagnosis of a neoplastic or hyperplastic disorder,
and may aid in detecting the presence of cancerous cells in the sample, e.g. Wilm's
tumor cells, by detecting cells in which a lesion of the WTI -BP gene has occurred or in
which the protein is. . .
. . .

IV, for example, encoding the
par-4 gene, may be useful for prediction of cell transfon-nation,
associated with potential
for a predisposition for cancer.

. . .
genomic WTI-BP sequences, can be used for both predictive
and therapeutic evaluation of allelic mutations which might be manifest
in, for example,
neoplastic or hyperplastic disorders (e.g. unwanted cell
growth) or abnormal
differentiation of tissue. The nucleotide probes can facilitate
determination of the
presence of. . .

. . .
56 -
The role of each of the subject WTI-BP in growth and differentiative
events,
such as those giving rise to Wilm's tumor, as well as normal
cellular functions of each of
the subject WTI-binding proteins, e.g. in regulation of transcription,
can be investigated
by. . .

. . .
ice. Extracts were incubated with WTI
- 65 -
antibodies (C19, Santa Cruz Biotechnology Inc., CA) or normal rabbit
serum (NRS)
overnight and immune complexes were collected with protein
A-Sepharose beads at 40
C for I hr. The beads were washed eight times with lysis. . .

. . .
1712; and Seto et al. (1991) Nature 345-:241-245). Total RNAs were
isolated from
these tissues (mouse) and from a human Wilms' tumor sample.
These RNA samples,
- 74 -
and RNA isolated from HeLa cells, 293 embryonic kidney cells, and K562
erythroleukernia cells, were probed. . .

Ciao-I mRNA was detected in kidney, testis, the Wilms' tumor
sample and
HeLa cells. Expression of Ciao-I was also detected in 293 embryonic
kidney cells and
K562 erythroleukemia cells, cell lines where. . .

(1992) Cancer Res. 52:6407-6412). Therefore, adult human
polyA-]- mRNA from a
1 5 variety of tissues was analyzed for WTI expression by Northern. .
.

. . .
activation and also potentiates repression functions of WTI. Further,
both
activation and repression functions of WTI have been implicated in its
tumor suppressor
1 5 functions (Haber, D. A. et al. (1993) Science 262:2057-2059; Park,
S. et al. (1993)
Cancer Res. 53:4757-4760; Reddy, J. C. et al. (I 995) J Biol.
Chem. 270: 10878-10884).

. . .
addition to the WTI gene on chromosome I IpI 3, WT2 on I IpI 5.5 has
also
been implicated in Wilms tumorigenesis (Reeve, A. E. et al. (I
989) Mol. CeIL Biol.

9:1799-1803). Chromosomal events causing deletion of chromosome 16 or

duplication
of chromosome 12 have been correlated with Wilms tumor
(Austruy, E. et al. (I 995)
Genes, Chro. & Cancer. 14:285-294). Other genetic loci have
also been suggested to
contributetothedisease(Altura,R.A.etal.(1996)CancerRes
.56:3837-3841). These
findings indicate involvement of novel gene products. Mutation or
abnormal expression
of proteins such as par-4, which modulates both the transcriptional. .
. and growth
regulatory functions of WTI, could lead also to aberrant expression of
certain growth-
regulatory proteins, and thus contribute to Wilms tumor
formation.

CLMEN. . . protein
to a par-4 binding protein, comprising:
combining:
(i) a par-4 protein or a binding portion thereof specific for an
ERK5 protein or an annexin V binding protein;
(ii) and a par-4 binding protein which is an ERK5 protein or an
annexin V protein or the par-4 binding portion thereof,
in the presence and absence of the agent; and
- 102 -
determining the degree. . .

=> d his

(FILE 'HOME' ENTERED AT 15:30:48 ON 19 JUN 2006)

FILE 'PCTFULL' ENTERED AT 15:31:04 ON 19 JUN 2006
L1 2848 S ANNEXIN
L2 297 S L1/AB OR L1/TI OR L1/CLM
L3 98312 S CANCER? OR NEOPLAS? OR TUMOR?
L4 252 S L2 AND L3
L5 13 S L4 NOT PY>1998
L6 9 S LUNG AND L5
L7 71233 S IMMUNE OR HUMORAL OR IMMUNIZATION OR IMMUNITY OR IMMUNIZING
L8 4 S L7 AND L6

=> s l6 not l8
L9 5 L6 NOT L8

=> d ibib 1-5

L9 ANSWER 1 OF 5 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1998004294 PCTFULL ED 20020514
TITLE (ENGLISH): RADIOLABELED ANNEXINS
TITLE (FRENCH): ANNEXINES RADIOMARQUEES
INVENTOR(S): KASINA, Sudhakar;
RENO, John, M.;
FRITZBERG, Alan, R.;
TAIT, Jonathan
PATENT ASSIGNEE(S): NEORX CORPORATION;
UNIVERSITY OF WASHINGTON
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

	NUMBER	KIND	DATE
	WO 9804294	A2	19980205

DESIGNATED STATES
W: CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT

SE
APPLICATION INFO.: WO 1997-US12977 A 19970724
PRIORITY INFO.: US 1996-8/690,184 19960726

L9 ANSWER 2 OF 5 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1996017618 PCTFULL ED 20020514
TITLE (ENGLISH): RADIOLABELED ANNEXIN-GALACTOSE CLUSTER CONJUGATES
TITLE (FRENCH): CONJUGUES GROUPES ANNEXINE-GALACTOSE RADIOMARQUES
INVENTOR(S): THEODORE, Louis, J.;
KASINA, Sudhakar;
RENO, John, M.
PATENT ASSIGNEE(S): NEORX CORPORATION
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9617618	A1	19960613

DESIGNATED STATES
W: CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1995-US15851 A 19951206
PRIORITY INFO.: US 1994-8/351,653 19941207
US 1995-PCT/US95/00953 19950123
US 1995-PCT/US95/07599 19950613

L9 ANSWER 3 OF 5 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1995034315 PCTFULL ED 20020514
TITLE (ENGLISH): RADIOLABELED ANNEXIN-GALACTOSE CONJUGATES
TITLE (FRENCH): CONJUGUES RADIOMARQUES D'ANNEXINE-GALACTOSE
INVENTOR(S): RENO, John, M.;
KASINA, Sudhakar
PATENT ASSIGNEE(S): NEORX CORPORATION
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9534315	A1	19951221

DESIGNATED STATES
W: CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1995-US7599 A 19950613
PRIORITY INFO.: US 1994-8/261,064 19940616
US 1995-PCT/US95/00953 19950123

L9 ANSWER 4 OF 5 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1995027903 PCTFULL ED 20020514
TITLE (ENGLISH): A METHOD FOR DETECTING AND/OR OPTIONALLY QUANTIFYING AND/OR SEPARATING APOPTOTIC CELLS IN OR FROM A SAMPLE
TITLE (FRENCH): PROCEDE DE DETECTION ET/OU DE QUANTIFICATION EVENTUELLE ET/OU DE SEPARATION DE CELLULES APOPTOTIQUES DANS OU DEPUIS UN ECHANTILLON
INVENTOR(S): REUTELINGSPERGER, Christiaan, Peter, Maria
PATENT ASSIGNEE(S): INSTITUUT BIOPRIME;
REUTELINGSPERGER, Christiaan, Peter, Maria
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9527903	A1	19951019

DESIGNATED STATES
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TT UA UG US UZ VN

KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC
NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.: WO 1995-NL134 A 19950411
PRIORITY INFO.: AT 1994-94200968.9 19940411

L9 ANSWER 5 OF 5 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1995019791 PCTFULL ED 20020514
TITLE (ENGLISH): RADIOLABELED ANNEXINS
TITLE (FRENCH): ANNEXINES MARQUEES ISOTOPIQUEMENT
INVENTOR(S): KASINA, Sudhakar;
DEWHURST, Timothy, A.;
RENO, John, M.;
TAIT, Jonathan;
STRATTON, John
PATENT ASSIGNEE(S): NEORX CORPORATION;
KASINA, Sudhakar;
DEWHURST, Timothy, A.;
RENO, John, M.;
TAIT, Jonathan;
STRATTON, John
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9519791	A1	19950727

DESIGNATED STATES
W: CA JP US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT
SE
APPLICATION INFO.: WO 1995-US953 A 19950123
PRIORITY INFO.: US 1994-8/185,660 19940124

=> d l8 ibib kwic 3-4

L8 ANSWER 3 OF 4 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1996003655 PCTFULL ED 20020514
TITLE (ENGLISH): METHOD FOR THE DETERMINATION OF THE PRETHROMBOTIC STATE
TITLE (FRENCH): PROCEDE POUR LA DETERMINATION D'UN ETAT PRETHROMBOTIQUE
INVENTOR(S): FREYSSINET, Jean-Marie;
ANTONI, Benedicte;
DONIE, Frederic;
LILL, Helmut
PATENT ASSIGNEE(S): BOEHRINGER MANNHEIM GMBH;
FREYSSINET, Jean-Marie;
ANTONI, Benedicte;
DONIE, Frederic;
LILL, Helmut
LANGUAGE OF PUBL.: German
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9603655	A1	19960208

DESIGNATED STATES
W: JP US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1995-EP2846 A 19950719
PRIORITY INFO.: AT 1994-94111514.9 19940723

DETD . . . (McNeil et al.,
Immunology and clinical importance of antiphospholipid antibodies. Adv.
Immunol. 49
(1991), 193 - 280). In addition, some tumorigenic cells
express higher amount of
3

phosphatidyl serine in their outer membrane leaflet than their differentiated non-tumorigenic
counterpart (Connor J. et al., Differentiation-dependent expression of phosphadityl serine in
mammalian plasma membranes: Quantitative assessment of outer leaflet lipid by pro-. . .

Some auto-immune disorders and other diseases, for example infection, inflammation,
neoplasia myocardialinfaction strokes or transient ischaemic attacks, venous thrombosis,
arterial thrombosis, pregnancy screening, connective tissue disease, thrombocytopenia, oral
contraceptive therapy, migraine/. . .
. . .
could be helpful for the diagnosis of
high levels of in vivo apoptosis associated with major diseases such as AIDS, cancer,
autoimmune disorders or artherosclerosis. The level of circulating apoptotic bodies could be
indicative of the development or evolution of the. . .
. . .
to determine circulating
apoptotic bodies of various origin which are associated with diseases leading to cell damage
for example AIDS, cancer, autoimmune disorders or
artherosclerosis. Furtheron, by the
term microparticles it is understood to include these apoptotic bodies of various origin
. . .
2.5 99.5 179
45
B...F. Lupus 3.83
1.65 97 174
?
F...C. Alcoholic intox., polyncvritis, ?
1.4 96.1 173
272,5
anti-MAG
K...0. Canccr, Metastasis (lung?), Heart
2.05 1.4 94
169 7
transplantation

M...11. Lupus 1.6
1 77 138
7
... M. Transplantation (Cyclosporin, 1.98
1.75 58 104. . . ?
W...J. Non-inflammatory heart failure 2.21
2.5 42 76
?
F...M. SLE, APL syndrome, fetal loss ?
2 39.2 70,5
68,3
H...M.-A. Cancer (ovary)
1.85 1.35 33
59 7
V...N. Not determined, at hospital admission ?
4.5 32.8 59
389,4
r
n, M...M-M. Lupus
2.17. . .

CLMEN 2. The method of claim 1, wherein the specific receptor in step (a) is annexin V.

11. The method of claim 10, wherein the specific receptor is an annexin-V-coated particle.

. . .

method of claim 23 wherein the circulating microparticies and/or stimulated procoagulant cells or synthetic phospholipid-containing liposomes are bound to an annexin-V-coated solid phase.

31. A method for the diagnosis of diseases associated with cell damage or cell death such as AIDS, cancer or paroxysmal nocturnal hemoglobinuria by determining the circulating apoptotic bodies.

SUBSTITUTE SHEET (RULE 26)

L8 ANSWER 4 OF 4 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER:	1995015979 PCTFULL ED 20020514
TITLE (ENGLISH):	PRETARGETING METHODS AND COMPOUNDS
TITLE (FRENCH):	PROCEDES ET COMPOSES DE PRECIBLAGE
INVENTOR(S):	THEODORE, Louis, J.; MEYER, Damon, L.; MALLETT, Robert, W.; KASINA, Sudhakar; RENO, John, M.; AXWORTHY, Donald, B.; GUSTAVSON, Linda, M.
PATENT ASSIGNEE(S):	NEORX CORPORATION
LANGUAGE OF PUBL.:	English
DOCUMENT TYPE:	Patent
PATENT INFORMATION:	

NUMBER	KIND	DATE
WO 9515979	A1	19950615

DESIGNATED STATES W:	CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.:	WO 1994-US14174 A 19941207
PRIORITY INFO.:	US 1993-8/163,188 19931207

DETD Background of the Invention

Conventional cancer therapy is plagued by two problems. The generally attainable targeting ratio (ratio of administered dose localizing to tumor versus administered dose circulating in blood or ratio of administered dose localizing to tumor versus administered dose migrating to bone marrow) is low.

Also, the absolute dose of radiation or therapeutic agent delivered to the tumor is insufficient in many cases to elicit a significant tumor response.

Improvement in targeting ratio or absolute dose to tumor is sought.

. . .

Such pretargeting methods are characterized by an improved targeting ratio or increased absolute dose to the target cell sites in comparison to conventional cancer therapy.

Cytokines, such as interleukins (e.g., IL-2 and

IL-4), colony stimulating factors (e.g., GM-CSF), interferons, (e.g., interferon-gamma), and tumor necrosis factor (TNF), may be employed as anti-tumor active agents in the practice of two-step or three-step pretargeting protocols of the present invention.

Figure 2 depicts radiorhenium tumor uptake in a three-step pretargeting protocol, as compared to administration of radiolabeled antibody (conventional means involving antibody that is covalently linked to chelated radiorhenium).

Figure 3 depicts the tumor uptake profile of NR-LU streptavidin conjugate (LU StrAv) in com-carison to a control profile of native NR-LU-10 whole a_ntibody.

Figure 4 depicts the tumor uptake and blood clearance profiles of NR-LU streptavidin conjugate.

Tumor is used as a prototypical example of a target in describing the present invention.

Active Agent: A diagnostic or therapeutic agent (the payload), including radionuclides, drugs, anti-tumor agents, toxins and the like. Radionuclide therapeutic agents are used as prototypical active agents.

Blood Tumor
I r slow
2)
00
R rapid
3)
-i r
Targeting moiety
Anti-ligand
D Ugand
FWJ Ugand-active agent
Binding site (i.e., receptor, antigenic determinant)
Uver
Kidney
Briefly, this three-step pretargeting protocol features administration of an. . .
. . .
result from
alteration in the affinity by subsequent binding of another moiety to the targeting moiety-ligand, e.g., anti-idiotype monoclonal antibody binding causes removal of tumor bound monoclonal antibody.

The targeting moiety of the present invention binds to a defined target cell population, such as tumor cells. Preferred targeting moieties useful in this regard include antibody and antibody fragments, peptides, and hormones. Proteins correspcnding to known cell surface receptors. . .
. . .
of the
antibody or polypeptide in the host recipient, permitting an increase in the half-life and a reduction in the possibility of adverse immune reactions.

Types of active agents (diagnostic or therapeutic) useful herein include toxins, anti-tumor agents, drugs and radionuclides. Several of the potent toxins useful within the present invention consist of an A and a B chain. The. . .

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cell surface receptor and creating a pore in cell membranes. The palytoxin structure is known and is described in Bignami et al., Cancer Research, 52:5759-5764, 1992.

Alternatively, a prodrug (i.e., inactive) form of palytoxin, such as N-W-hydroxyphenylacetyl)-palytoxin described in Bignami et al., Cancer Research, 52: 5759-5764, 1992, and activated by penicillin G amidase (PGA) , may be employed in two-step or three-step pretargeting protocols of. . .

. . .

include
conventional chemotherapeutics, such as vinblastine, doxorubicin, bleomycin, methotrexate, 5-fluorouracil, 6-thioguanine, cytarabine, cyclophosphamide and cis-platinum, as well as other conventional chemotherapeutics as described in Cancer: Principles and Practice of Oncology, 2d ed., V.T. DeVita, Jr., S.

. . .

as Pseudomonas exotoxin (PE)
and trichothecenes, are primarily metabolized in the liver. Consequently, liver toxicity is associated with administration of PE. Also, administration of anti-tumor agents, such as IL-2 and TNF, has been shown to result in liver toxicity. Active agents characterized by such a biodistribution pattern. . .

. . .

be given. Because of the
high affinity of ligand for the complementary anti-ligand, a therapeutically effective dose may be delivered to the tumor, without the necessity for active agent-targeting moiety binding during targeting moiety accretion to target site. The active agent-ligand or active agent-anti-ligand are. . .

other anti-tumor agents,e.g., agents active against proliferating cells, are administrable in accordance with the present invention. Exemplary anti-tumor agents include cytokines and other moieties, such as interleukins (e.g.,IL-2, IL-4, IL-6, IL-12 and the like), transforming growth factor-beta, lymphotoxin, tumor necrosis factor, interferons (e.g., gamma-interferon), colony stimulating factors (e.g., GM-CSF, M-CSF and the like), vascular permeability factor or the like, lectin inflammatory response promoters. . .

Also, suitable anti-tumor agents include compounds which inhibit angiogenesis and therefore inhibit metastas.is. Examples of such moieties include protamine and platelet factor 4 (described in U.S.

. . .

No. 5,284,827). These compounds are also useful for treatment of diseases involving angiogenic dysfunctions such as diabetic retinopathy, retrolental fibroplasia, neurovascular glaucoma, psoriasis, angiofibromas, immune and non-immune inflammation, capillary proliferation with atherosclerotic plaques,

hemangiomas and Kaposils Sarcoma.

One problem with this method, however, is the potential for cross-linking and internalizing tumor-bound biotinylated antibody by avidin.

Nucl. Med. 33(10):1816-18, 1992). Poly-bictinylated transferrin also has the potential for cross-linking and internalizing tumor-bound avidinylated-targeting moiety, however. In addition, both chase methodologies involve the prolonged presence of aggregated moieties of intermediate, rather than large, size (which are not. . .

. . .

receptor, a liver specific degradation system, as opposed to aggregating into complexes that are taken up by the generalized RES system, including the lung and spleen. Additionally, the rapid kinetics of galactose-mediated liver uptake, coupled with the affinity of the ligand-anti-ligand interaction, allow the use of intermediate. . .

. . .

is to be excluded. For example, high molecular weight (ranging from about 200,000 to about 1,000,000 Dal) clearing agents will be used when tumor targets or clot targets are involved.

. . .

to more effectively deliver a therapeutic or diagnostic dose of radiation to a target site, the radionuclide is preferably retained at the tumor cell surface. Loss of targeted radiation occurs as a consequence of metabolic degradation mediated by metabolically active target cell types, such as tumor or liver cells.

The patterns of cross-reactivity for monoclonal antibodies directed against a particular target site are analyzed to identify a set of two or. . . known to be preserved following fixation, such a technique may be effectively used. in vitro histochemical procedures are known (e.g., Ceriani et al., Cancer Research, 47: 532-540, 1987 or Example I of U.S.

U.S. Patent No. 51284,934 to Allen, Jr. similarly describes the preparation of carbohydrate-binding lectin derivatives for use as immune modulators or immune conjugates by attachment of polyethylene glycol to Ricinius communis agglutin I (PEG-RCAI) to a polymer by use of a coupling agent, e.g.,. . .

. . .

further known that PEG modified protectors are resistant to metabolic deactivation. The following references are representative of PEG modification of proteins. Wang et al., Cancer Res., 53, 4588-4597 (1993), who describe PEG attachment to a chimeric toxin; Rosenberg et al., J. Biol. Chem., 267 (32), 2289-2293 (1992), who. . . (1992) who describe PEG modification of macrophage colony stimulating factor (GM-CSF) to produce derivatives having conserved biological activity; and Tsutsumi et al., Jaipan J. Cancer Res., 85, 9-12 (1994) who describe PEG modification of tumor necrosis factor to produce conjugates having improved anti-tumor

activity. In some instances PEG modification of a protein has been disclosed to result in loss of biological activity. (See, e.g., Wang et. . .

. . .

primarily via renal excretion; and the superantigen will localize to the target cell site and thereby induce an inflammatory response of the recipient's immune system.

Superantigens refer to highly immunogenic molecules that are capable of inducing an immune response in a recipient without the necessity for internalization and antigen presentation. The prototypical superantigen discussed herein is staphyloccal enterotoxin A. Superantigens are known to. . .

Highly immunogenic molecules that are capable of inducing an immune response in a recipient without internalization and antigen presentation (superantigens) are useful in the practice of the present invention. Exemplary superantigens are bacterial and mycoplasma exoproteins, such as the staphylococcal and streptococcal exotoxins, and an exoprotein produced by M ycoplasma arthritidis, viral antigens such as the mammary tumor virus encoded Mla antigens and the like. Other superantigens are also well known in the art. See, e.g., the following review articles pertaining. . .

. . .

of heart attack victims. t-PA exhibits several advantages over other thrombolytics. Because it is of human origin, it is less subject to adverse immune reactions. This is in contrast to streptokinase which, while it efficiently dissolves clots, is of bacterial origin and therefore may mediate an immune reaction. Also, t-PA is more specific in its action than other thrombolytic therapeutic agents, e.g., urokinase and streptokinase.

Another problem associated with some thrombolytics is that they may mediate an immune reaction which neutralizes the activity of the administered therapeutic protein. As discussed, this is a particular concern for streptokinase given its bacterial origin. However,. . . enhanced clot dissolving activity, affinity and/or stability, the fact that they are mutant proteins increases the likelihood that they may produce an adverse immune response upon is administration, particularly if the particular derivative is to be used for a prolonged time period or repeated usage.

Also, such clot dissolving agents may stay in the immune circulation after they have mediated the desired therapeutic effect, i.e., dissolved a blood clot. This also enhances the potential that such administration may. . .

Gene therapy methods are particularly promising for the treatment of cancer. Such methods, for example, comprise the delivery of nucleic acid secruences which activate the host immune system to recognize antigens expressed on the cancer cells as

foreign, and thereby activate the immune system to attack the tumor. Examples of such nucleic acid sequences include genes which encode Class 1 transplantation antigens. Nucleic acid sequences which encode cytokines, e.g., interleukins such as IL-1, IL-2, interferons, tumor necrosis factor, are also promising candidates for treatment of cancer by gene therapy. Additionally, the delivery of nucleic acid sequences which are cytotoxic to the cancer cell, e.g., anti-tumor agents, cytotoxins, or which disrupt an essential cell function show promise in such gene therapies.

However, while gene therapy is a promising tool for the treatment of cancer, it suffers from one serious disadvantage. In particular, conventional gene therapy methods typically require direct injection into the cells which are to be treated. In the case of cancer treatment, this entails the direct injection of the therapeutic nucleic acid sequence, for example, a plasmid, into surface cancer lesions.

This is disadvantageous because it limits the potential efficacy of such gene therapies to surface cancers. Accordingly, a method of gene therapy which enables nucleic acid sequences, e.g., plasmids, to be targeted and introduced into cancer cells by systemic injection would be highly beneficial since it would enable gene therapy to be useful for treatment of a variety of different cancers.

. . .
present inventors have developed a method whereby desired nucleic acid sequences, e.g., which are contained on a plasmid may be effectively targeted to cancer cells by systemic injection. In particular, this will be effected by.

. . .
(i) a pretargeting step comprising administering a ligand or anti-ligand bound to a targeting moiety specific to an antigen expressed on the cancer cells which are to be targeted; and
(ii) administration of an active agent which comprises a nucleic acid sequence which upon delivery and stable insertion in said cancer cells imparts a therapeutic effect, and wherein said nucleic acid sequence is associated with a ligand or anti-ligand which binds the ligand or. . .

. . .
attached to the ligand or anti-ligand. In the preferred embodiment, the nucleic acid sequence, e.g., plasmid, which is to be targeted to the cancer cells will be encapsulated in a liposome which is in turn attached to the particular ligand or anti-ligand and therefore specifically binds. . .

. . .
Moreover, the administration of liposome enca-osulated nucleic acid sequences has also been reported to be efficacious for the treatment of melanoma, a surface cancer. For example, it was reported in a recent Bioworld Toda , Vol. 4, No. 233, 1 and 5, that a cDNA encoding. . . plasmid, wrapped in a liposome-like sheath, and injected into the lesions of a melanoma patient, caused the effective regression of metastases in the lungs and the lysing of local skin nodules remote from the injection sites. However, as

promising as these results are, it is still disadvantageous in the fact that efficacy requires injection into cancer lesions.

. . .

by a pretargeting step,, which comprises administration and delivery of a ligand- (or anti-ligand) targeting moiety conjugate to the targeted sites, i.e., tumor cells, followed by the administration of the nucleic acid sequence (e.g., plasmid) containing liposome, wherein said liposome is attached to a ligand or. . .

. . .

encapsulated
nucleic acid sequence when used in pretargeting methods since it should facilitate the efficient delivery of such liposome to the targeted site, i.e., tumor cells.

. . .

to a molecular weight of ==
io8,ooo. with such a size and molecular weight, the liposome containing plasmid should localize effectively on the tumor and be taken up by the reticuloendothelial system (RES). Such plasmids will then he internalized into the targeted cell, wherein the nucleic acid. . .

Because of such problems, if the targeted site, e.g., a tumor, contains a large number of hypoxic cells, it may be difficult to administer a sufficient dosage of the radioactive active agent or chemotherapeutic. . . bU]
killing of hyp] _L I which does not cause adverse effects to normal tissues. Such problems are particularly prevalent for solid tumors which, given their morphology, tend to contain greater numbers of hypoxic cells.

. i) be capable of rapid transfer to dense cell populations characterizing hypoxic tumor cells or to the vasculature thereof;
ii) exhibit favorable residence time in a mammalian system, i.e., not be too rapidly eliminated by excretion, transpiration. . .

Ideally, such compounds, if used systemically diffuse quickly through the vasculature, pick up oxygen in the lungs, remain in the cardiovascular system for a sufficient time during therapy, and then are rapidly eliminated without undue toxicity.

Phys.,, 22, 87-93 (1992). Also, hemoglobin enhances oxygen delivery as a protein solution (J. Cancer Rev.

Preferably, administration will be systemic and at a site enabling the dispersion to traverse the lungs to pick up oxygen and to transport the oxygen to the hypoxic tumor cells. Dosages of the dispersion will be predetermined in accordance with the site and character of the hypoxia, whether or not the. . .

Contact of the PFC dispersion may be with the hypoxic cells or with the tumor cell vasculature, such that the oxygen carried by the PFC may transfer to the tumor/vasculature interface. In other words, while the ideal may be direct contact between the PFC

dispersion and the hypoxic cells, this may not be achievable and in fact is not required, since excess oxygen, wherever present in the tumor mass, will tend to become distributed throughout the mass, and thus reach the hypoxic cells.

. . .

prior to
irradiation and/or chemotherapy will also be controlled by various conditions, including the rate at which the perfluoro compound travels to the hypoxic tumor cells, the degree of sensitization desired, and .the cardiovascular half-life residence time of the dispersion in the cardiovascular system and in the hypoxic. . . can be as brief as about 2 to 4 hours. This duration indicates that the perfluoro compound moves rapidly to the hypoxic tumor cells and transfers its oxygen to the cells. In this connection an outstanding property of the preferred dispersions of the invention is an. . .

. . .

be
expedient when injection is at or near the site of the hypcxJa rather than at a site where oxygen transfer from the lungs and arteries is anticipated. Prior oxygenation in such manner may be accomplished by any means, such as flushing or blanketing a vessel containing. . . case
of preoxygenation, however, there may be a loss of oxygen prior to entry of the dispersion into the region of the hypoxic tumor cells, that, during transit of the dispersion to the cells; hence, preoxygenation generally is not preferred.

The sensitizing method when used in the pretargeting methods of the present invention will be effective for all types of hypoxic tumor cells, whether such cells be in suspension (as in leukemia) or in solid form, but the invention is particularly effective for solid tumors. Because systemic distribution of the dispersions is rapid, primarily due to the extremely small and stable particle size of the preferred dispersions of. . .

Chemotherapy is often used in combination with radiotherapy to destroy or control hypoxic tumor cells and therefore the sensitization techniques of the invention can be applied simultaneously or sequentially to chemotherapy and radiotherapy. When dual therapy is used,. . .

. . .

as
aminoethylisothiuronium or the phosphorothioate derivatives of betamerda-Qtoethylamine reviewed in the article by J.M. Yuhas, On the Potential AnDlication of Radioprotective Drugs in Solid Tumor Radiotherapy, appearing in Radiation-Drug Interactions in the Treatment of Cancer, edited by G.H. Sokol and R.P.

. . .

(1980), pp. 113-
13S. Another RP agent is S (3-aminopropylamino) ethylphosphorothioic acid, also described in the article by Yuhas et al. appearing in Cancer Clinical Trials, (1980), 3, 211
other suitable sensitizers include fluorocarbon emulsions which comprise at least one perflucrocarbon

compound having 9 to 11 carbon. . .

. . .

identified sensitizers promote the
efficacy of radioactive compounds and chemotherapeutic agents by providing for enhanced delivery of oxygen to the targeted site, e.g., tumor cells, thereby enhancing the activity of such active agents against hypoxic cells. The present invention further.

. . .

by target cell
biochemistry (i.e., photosensitizing agents soluble at low pH will be retained longer by target cells exhibiting low pH such as tumor cells); or by the hydrophobicity of the photosensitizing agent (greater hydrophobicity enhances retention).

. . .

nm) can act on target sites embedded more deeply in tissue than photosensitizing agents absorbing light of lower wavelengths. while initially only skin cancers were treated with photodynamic therapy, the conventional procedure has now been applied to early stage tumors in the head and neck, brain, lung, gastrointestinal and genitourinary tracts.

. . .

as a therapeutic agent when associated
with target bound conjugate. In contrast to the internalization caused by cross-linking described above, cross-linking at the tumor cell surface stabilizes the monovalent fragment-anti-ligand molecule and, therefore, enhances target retention, under appropriate conditions of antigen density at the target cell. In addition,. . .

. . .

preferably
administered in an amount which provides for the requisite therapeutic amount of the active moiety to reach the target site, e.g., a tumor, but which does not result in undue toxicity to normal tissues. Such toxicity is a potential concern if the particular active agent is. . .

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for
which supply arteries are accessible. Exemplary applications for intraarterial delivery aspects of the pretargeting methods of the present invention include treatment of liver tumors through hepatic artery administration, brain primary tumors and metastases through carotid artery administration, lung carcinomas through bronchial artery administrat4on and kidney carcinomas through renal artery administraticn.

Intraarterial administration pretargeting can be conducted using chemotherapeutic drug, toxin and anti-tumor active agents as discussed below. High potency drugs, lymphokines, such as IL-2 and tumor necrosis factor, gamma-interferon, drug/lymphokine-carrier-biotin molecules, biotinylated drugs/lymphokines, and drug/lymphokine/ toxin-loaded, bictin-derivatized liposomes are exemplary of active agents and/or dosage forms useful for the delivery. . .

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in combination with
delivery of a radionuclide diagnostic or therapeutic moiety, the protocols are amenable to use for delivery of other moieties, including anti-tumor agents,

chemotherapeutic drugs and the like. For example, most naturally occurring and recombinant cytokines have short in vivo half lives. This characteristic limits the. . . of these molecules, because near toxic doses are often required. Dose-limiting toxicities in humans have been observed upon high dose IL-2 or tumor necrosis factor, gamma-interferon or lymphotoxin administrations, for example.

Anti-tumor agents, such as IL-2 and TNF, may be employed as active agents in the practice of two-step or three-step pretargeting protocols of the present invention. Some anti-tumor agents exhibit short circulation half-lives (less than about 1 hour post-administration), such as IL-2 (half-life of about 10 minutes), other interleukins, TNF,. . .

Anti-tumor agents having longer half-lives (ranging from about 2 hours to about 12 hours post-administration) are preferably employed at low doses, while conjugates. . .

Ligand or anti-ligand de.rivat-izaticn of a long half-life anti-tumor agent may decrease the serum half-life sufficiently to permit higher doses of conjugate to be administered, however.

. . .
device or an
alternative clearing procedure to substantially remove circulating conjugate from the recipient; and administering to the recipient a second conjugate comprising an anti-tumor agent, such as IL-2, and a ligand/anti-ligand binding pair member, wherein the second conjugate binding pair member is complementary to that of the. . .

Concentrations of IL-2 effective in maintaining activated tumor infiltrating lymphocytes (TIL) and for other forms of anti-tumor therapy have also been found to be toxic to the recipient. Localization of IL-2 to is the tumor microenvironment allows localized activation of effector cells to poorly immunogenic tumor antigens. Effector cells of the cytotoxic T-lymphocyte lineage and T-helper lineage are induced to recognize tumor antigens and clonally expand to seek out tumor metastases at other locations.

Furthermore, B cells may be induced to secrete antibody specifically recognizing tumor antigens.

These B cells mature into IgG secretory cells and memory cells and continue to expand when tumor antigens are detected at other sites. In addition, natural killer cells are candidates for anti-tumor immunity, because such cells are also activated by IL-2. Studies in SCID-beige mice deficient in T and B cells but NK competent showed that such mice were effective hosts in rejecting an IL-2 transfected tumor cell line. See Alosco et al., Cancer Immunol.

Tumor necrosis factors (TNFs) have been isolated from a variety of mammalian species. For example, human, murine, rabbit and guinea pig exhibit at. . .

TNF receptor-bearing cells include adipocytes, myotubes, cervical carcinoma, fetal lung, bladder carcinoma, histocytic leukemia, erthroleukemia, promyelocytic leukemia, epidermoid carcinoma, cervical carcinoma, T lymphoma, human lymphocytes, lymphoblastic leukemia (two receptors), monocytic leukemia, foreskin fibroblast, connective tissue. . . . . .

TNF may be delivered to target cells essentially]in the manner described above for IL

TNF itself is cytotoxic to a narrow spectrum of tumor cells; however, this cytokine exhibits a broad range of immunologic modulating activities. one such activity is activation of tumor infiltrating macrophages or monocytes, thereby rendering the macrophages tumoricidal. One theory regarding the mechanism of TNF in this regard suggests that TNF stimulates monocytes to progress to macrophages which are, in turn,. . . TNF to release cytotox-4c factors (e.g., oxidative burst or protease secretion or cytokine release). TNF release by activated macrophages can maintain or induce tumoricidal activity through an autocrine mechanism. Additional activation of monocytes or macrophages by other cytokines (e.g., gamma-interferon) may be employed to enhance the cytotoxic effect.

. . . of monocytes with recombinant TNF-alpha for 1 hour followed by treatment of medium alone or gamma-interferon led to increased killing of the aforementioned tumor cell types by the TNF-alpha/gamma-interferon-treated membrane preparations of monocytes. See, for example, Peck et al., Cellular Immuncl., 132: 308, 1991).

899 9000, 1993, discuss the concept of vascular targeting. Tumor target vascular endothelial cells are accessible to circulating agents. Burrows et al.

. . . transfected cells secreted INF-gamma, which induced expression of class II antigens of the major histocompatibility complex by capillary and venular endothelial cells within the tumor mass. An immunotoxin targeted to such MHC class II antigens was then administered and rapid accretion to tumor and tumor regressions were observed. Relapses wbre observed 7-10 days after treatment and were attributed to surviving tumor cells that derived nutrition from the extratumoral blood supply.

administering to the recipient a first conjugate comprising a targeting moiety specific for tumor endothelial cells and INF-gamma, wherein the first conjugate localizes to a target site and the INF-gamma induces expression of MHC class II antigens by tumor endothelial cells;

optionally administering to the recipient a clearing agent capable of directing the clearance of circulating conjugate from the recipient or optionally is treating. . . device or an alternative clearing procedure to substantially remove circulating conjugate from the recipient; and

administering to the recipient a second conjugate comprising an anti-tumor agent, such as a toxin, a radionuclide, an anti-tumor agent or the like, and a targeting agent specific'for MHC class II antigens.

administering to the recipient a first conjugate comprising a targeting moiety specific for tumor endothelial cells and a member of a ligand-anti-ligand binding pair, wherein the first conjugate localizes to a target site;
optionally administering to the recipient. . . device or an alternative clearing procedure to substantially remove circulating conjugate from the recipient; and
administering to the recipient a second conjugate comprising an anti-tumor agent, such as a toxin, a radionuclide or an anti-tumor agent, and a ligand/anti-ligand binding pair member, wherein the second conjugate binding pair member is complementary to that of the first conjugate.

This latter protocol offers the advantages of not relying on tumor endothelial cells to express the antigen recognized by the active agent-bearing conjugate and of not exposing the recipient to systemically administered INF-gamma.

Also, an optional additional step is the administration of a conjugate incorporating a targeting moiety specific for tumor cells and a cytotoxic active agent. Alternatively, administration of a conjugate comprising a targeting moiety specific for.tumor cells and the member of the ligand-anti-ligand binding pair incorporated in-the first conjugate, wherein this conjugate localizes to a target site. In this manner, tumor cells that receive nutrition from the extratumoral vasculature can be addressed.

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It is another object of the invention to increase the utilization of cytokine (ligand or anti-ligand) conjugates at targeted sites, e.g., a tumor. More particularly, it is an object of the invention to increase the utilization of TNF-biotin/(streptavidin or avidin)-antibody conjugate system as a delivery system for delivery of cytokines to tumor cells. This is accomplished by providing for the site specific release of biologically active, free cytokine, e.g., TNF at the targeted site.

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complex
of NR-LU-10/streptavidin precomplexed with biotinylated TNF. This premadell fusion construct is an effective means for site-sDecifically delivering a cytokine, e.g., TNF, to a tumor site. Moreover, it is believed that this may decrease systemic toxicity associated with TNF.

In this regard, the utilization of cytokines, including tumor necrosis factor (TNF), has been limited by both their systemic toxicity and the lack of effective targeting to desired sites of action.

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secreted by
mononuclear phagocytes in response to stimulation by bacterial endotoxin and other agents. It has been

shown to produce hemorrhagic necrosis of tumors in vivo. The delivery of TNF to target sites is plagued by its short half-life (11-30 min. in humans) and its toxic effects. . . in
septic shock. Pretargeting has been shown to be an effective methodology for trapping molecules with a relatively short serum half-life at the tumor, allowing their accretion to high concentrations within a short time. These molecules are then retained for long periods of time due to slow off-rate and/or diffusion of the antibody-streptavidin conjugate from the tumor.

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of free TNF-Bt. Since TNF is a
protein of approximately 16 kD molecular weight, it should not diffuse away rapidly from the tumor, therefore allowing an effective local concentration to be achieved.

Other anti-tumor agents that may be delivered in is accordance with the pretargeting techniques of the present invention are selectins, including L-selectin, P-selectin and E-selectin.. . .

While the majority of the exemplary pretargeting protocols set forth above have been directed to the treatment of cancer, pretargeting protocols may be employed in the diagnosis or treatment of other conditions. Exemplary additional conditions are is discussed below.

For example, delivery of active agents, such as interferon-gamma, tumor necrosis factor-alpha or a combination thereof, to monocytes or macrophages or tumor cells via pretargeting protocols will facilitate the treatment of cancer through the mechanism of activating the monocytes to cytotoxic macrophages.

Activated macrophages excrete toxic moieties such as enzymes, lysozyme cathepsins and hydrolases. Tumor necrosis factor-alpha acts via activation of cytotoxic macrophages, direct tumor cell killing or rendering the tumor cells more susceptible to effector cell-mediated cytotoxicity. A combination of the two active agents serves to optimally prime macrophage tumoricidal activity. Targeting to the target tissues may be accomplished via antibodies directed to tumor specific antigens, such that the active agents are presented to the infiltrating monocyte population in a localized environment.

For example, delivery of active agents, such as interleukin-2, to cytotoxic T-cells via pretargeting protocols will facilitate the treatment of cancer through the mechanism of activating tumor infiltrating lymphocytes (TIL). Killing by cytotoxic T-cells is believed to be mediated by release of pore-forming moieties that insert into the membrane of the target cell and facilitate target cell lysis. Targeting to the target tissues may be accomplished via antibodies directed to tumor specific antigens.

. . .
active agents, such as
granulocyte monocyte colony stimulating factor (GM-CSF), to macrophages and polymorphonuclear neutrophils

via pretargeting protocols will facilitate the treatment of cancer through the mechanism of activating infiltrating monocytes and polymorphonuclear leukocytes (PMNs). Killing by monocytes and polymorphonuclear leukocytes is the result of cytotoxic enzymes released by activated forms of these cells. Targeting to the target tissues may be accomplished via antibodies directed to tumor specific antigens.

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or other targeting
moiety which recognizes fibroblast activation protein or antigen 19 on stimulated stromal fibroblasts. See, for examp le, Rettig et al., Cancer. Res., 53: 3327, 1993. In this manner, TGF-beta may be used to inhibit chronic inflammation by targeting stromal fibroblasts activated by peptide mediators. . .

. . .

such as
roridin A, verrucarin A, anguidine and like trichothecenes to RNA via pretargeting protocols will facilitate the treatment of insulin-dependent diabetes mellitus or cancer through the mechanism of protein synthesis inhibition. Targeting to the target tissues may be accomplished via antibodies directed to tumor specific antigens or to the pancreas.

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in human clinical trials. The hybridoma secreting NR-LU-10 was developed by fusing mouse splenocytes immunized with intact cells of a human small cell lung carcinoma with P3 x 63/Ag8UI murine myeloma cells. After establishing a seed lot, the hybridoma was grown via in vitro cell culture. . .

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profiles of NR-LU
The NR-LU-10 target antigen was present on either fixed cultured cells or in detergent extracts of various types of cancer cells. For example, the NR-LU-10 antigen is found in small cell lung, non-small cell lung, colon, breast, renal, ovarian, pancreatic, and other carcinoma tissues. Tumor reactivity of the NR-LU-10 antibody is set forth in Table A, while NR-LU-10 reactivity with normal tissues is set forth in Table. . .

The NR-LU-10 antigen has been further described by Varki et al., Antigens Associated with a Human Lung Adenocarcinoma Defined by Monoclonal Antibodies, Cancer Research, 44: 681-687, 1984, and Okabe et al., Monoclonal Antibodies to Surface Antigens of Small Cell Carcinoma of the Lung, Cancer Research, 44.

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inert, insoluble
matrix capable of binding immunoglobulin, thereby forming an immunosorbent;
- combining the immunosorbent with an extract containing polyvalent NR-LU-10 antigen, forming an insolubilized immune complex wherein the first epitope is masked by the first monoclonal antibody;
is - immunizing an animal with the insolubilized immune complex;
- fusing spleen cells from the immunized animal to myeloma cells to form a hybridoma capable of producing a second monoclonal

antibody directed. . .

Table A
TUMOR REACIT= OR ANTIBODY NIR-LU-10
Organ/Cell Type #Pos/ Intensitya Percenth Umforrruryc
Tumor Exam Avg. Range Avg. Range Avg. Range
Pancreas Carcinoma 6/6 3 3 100 100 2.3 2-3
Prostate Carcinoma 9/9 I 2.8 2-3 95 80-100 2 1-3
Lung Adenocarcinoma 8/8 3 3 100 100 2.2 1-3
Lung Small Cell Carcinoma 212 3 3 100 100 2 1 2
Lung Squamous Cell Carcinoma 8/8 2.3 2-3 73 5-100 1.8 1-3
1 0 Renal Carcinoma 8/9 1 2.2 2-3 1 83 75-100. . .
. . .
0/3
Mesangial 0/3 0
Proximal Convoluted Tubules 3/3 500
0 Liver
Bile Duct 3/3 500
Central Lobular Hepatocyte 1/3 4
Periportal Hepatocyte 1/3 40
Kupffer Cells 0/3 0
Lung
Alveolar Macrophage 0/3 0
Bronchial Epithelium 0/2 0
Bronchial Smooth Muscle 0/2 0
Pneumocyte Type 1 3/3 354
Pneumocyte Type II 3/3 38
Lymph Node
Lymphoid Follicle-Central. . .
. . .
conjugate (3)
showed approximately 50']. reduction of radioactivity in
the intestines and 60]'. in the kidneys. The conjugate
(3) exhibited similar biodistribution in tumor bearing
mice for tumor uptake and radiclabel clearance from
the blood compared to the many other conjugates
suggesting that the serylsuccinate linkage is serum
stable.
. . .
a result of this cross-reactivity,
introduction of biotinylated ligand results in binding
to the streptavidin pretargeted to this site as well
as to tumor.
. . .
between the
radioactive chelate and biotin. This requires the
selection of a linker that is selectively cleaved in
normal tissue, but not in tumors. There has been
substantial investigation in this area. Ester linkages
have been reported to give higher tumor to blood ratios
(See, Haseman, M.K. et al., Eur. J. Nucl. Med.,
Metabolizable In-III chelate conjugated anti-idiotype
monoclonal antibody for radioimmunodetection of lymphoma
in. . . Hylarides et al.,
Bioconjugate Chemistry on PIP Ester-linked Fab
Conjugates; and Kasina, S., et al., The Third Conference
on Radioimmunodetection and Radicimmunotherapy of Cancer,
Princeton, NJ, 1990). The ester linked MAG 3 Fab'
conjugate 1, shown below, reportedly gave a two-fold
improvement in clearance from the kidney. . .
. . .
a
measured derivitization of 11.1:1; however, at this level
of derivitization, no decrease was observed in antigen-
positive cell binding (performed with LS-180 tumor cells

at antigen excess). Subsequent experiments used antibody derivatized at a bictin:antibody ratio of 10:1.

P. Blood Clearance of Bictinviated Antibcdv Snecies

Radiciodinated biotinylated NR-LU-10 (lysine or thiol) was intravenously administered to non-tumored nude mice at a dose of 100 Ag. At 24 h post-administration of radioiodinated biotinylated NR-LU-10, mice were intravenously injected with either saline. . .

More specifically, 18-22 g female nude mice were implanted subcutaneously with LS-180 human colon tumor xenografts, yielding 100-200 mg tumors within 10 days of implantation.

Third, tumor uptake of hictinylated antibody administered at time 0 or of avidin administered at time 24 h was examined. The results of this experimentation are shown in Fig. 1. At 25 h, about 350 pmcl/g bictinylated antibody was present at the tumor; at 32 h the level was about 300 pmol/g; at 48 hf about 200 pmol/g; and at 120 h, about 100 pmol/g. Avidin uptake at the same time points was about 250, 150, 50 and 0 pmol/g, respectively. From the same experiment, tumor to blood ratios were determined for biotinylated antibody and for avidin. From 32 h to 120 h, the ratios of tumor to blood were very similar. Rapid and efficient removal of biotinylated antibody from the blood by complexation with avidin was observed. Within two hours of avid-in administration, a 10-fold reduction in blood pool antibody concentration was noted (Fig. 1), resulting in a sharp increase in tumor to blood ratios. Avid-in is cleared rapidly, with greater than 90% of the injected dose cleared from the blood within 1 hour. . .

The three-step pretargeting protocol (described for Group 1, above) was then examined. More specifically, tumor uptake of the 'Re-chelate-bilotin conjugate in the presence or absence of bictinylated antibody and avidin was determined. In the absence of bictinylated antibody and avidin, the 186Re-chelate-bictin conjugate displayed a slight peak 2 h post-injection, which was substantially cleared from the tumor by about 5 h. In contrast, at 2 h post-injection in the presence of biotinylated antibody and avidin (specific), the ^{186}Re-chelate-bictin conjugate reached a peak in tumor approximately 7 times greater than that observed in the absence of biotinylated antibody and avidin. Further, the specifically bound 186Re-chelate-bictin conjugate was retained at the tumor at significant levels for more than 50 h. Tumor to blood ratios determined in the same experiment increased significantly over time (i.e., T:B a at 30 h;] 15 at 100 h;. . .

Tumor uptake of the 186 Re-chelate-biotin conjugate has further been shown to be dependent on the dose of biotinylated antibody administered. At 0 Ag of biotinylated antibody, about 200 pmol/g of 186Re-chelate-biotin conjugate was present at the tumor at 2 h after administration; at 50 Ag antibody, about 500 pmol/g of 186Re-chelate-biotin conjugate; and at 100 Ag antibody, about 1,300 pmol/g. . .

Rhenium tumor uptake via the three-step pretargeting protocol was compared to tumor uptake of the same antibody radiolabeled through chelate coValently attached to the antibody (conventional procedure). The results of this comparison are depicted in Figure 2. Blood clearance and tumor uptake were compared for the chelate directly labeled rhenium antibody conjugate and for the three-step pretargeted sandwich. Areas under the curves (AUC) and. . .

tumor blood
For the chelate directly labeled rhenium antibody conjugate, the ratio of AUC /AUC 24055/10235 or
tumor blood =
2.35; for the three-step pretargeted sandwich, the ratio of AUCtumor/AUCblood] 46764/6555 or 7
i 6 4,
Tumor uptake resa-zs are best taken in context with radioactivity ex=c.=,are to the blocd compartment, which directly correlates wi-.h bone marrow exposure. Despite the. . . greater exposure to rhenium than did the 100-fold higher dose given in the three-step protocol. A clear increase in the targeting ratio (tumor exposure to radioactivity:blood exposure to radioactivity--AUCtumor: AUC blood) was observed for three-step pretargeting (approximately 7:1) in comparison to the direct labeled antibody approach. . .

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of blood clearance by varying the molecular weight of the polylysine polymer; and (3) increasing the circulation half-life of the conjugate for optimal tumor interaction.

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Linkage
Through insertion of a cleavable linker between the chelate and bictin portion of a radiometal-chelate-biotin conjugate, retention of the conjugate at the tumor relative to normal tissue may be enhanced. More specifically, linkers that are cleaved by enzymes present in normal tissue but deficient or absent in tumor tissue can increase tumor retention. As an example, the kidney has high levels of T-glutamyl transferase; other normal tissues exhibit in vivo cleavage of T-glutamyl prodrugs.

In contrast, tumors are generally deficient in enzyme
-linked biotin conjugate
peptidases. The glutamyl
depicted below is cleaved in normal tissue and retained in the tumor.

1311-PIP-biocytin was evaluated in a two-step pretargeting procedure in tumor-bearing mice. Briefly, female nude mice were injected subcutaneously with LS-180
tumor cells; after 7 d, the mice displayed 50-100 mg
tumor xenografts. At t = 0, the mice were injected with 200 Ag of NR-LU streptavidin conjugate labeled with 125I using PIP-NHS (see Example IV.A.). At t = 36 h, the
Ag of 131,
mice received 42 -PIP-biocytin. The data showed immediate, specific tumor localization,
131
corresponding to = 1.5 I-PIP-biocytin molecules per avidin molecule.

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the

circulation. one of ordinary skill in the art of radioimmunoscintigraphy is readily able to determine the optimal time for NR-LU avidin conjugate tumor localization and clearance from the circulation. At such time, the 99mTc-chelate-biotin conjugate is administered to the recipient. Because the 99mTc-chelate-biotin conjugate has a molecular weight of] 1,000, crosslinking of NR-LU avidin molecules on the surface of the tumor cells is dramatically reduced or eliminated. As a result, the 99mTc diagnostic agent is retained at the tumor cell surface for an extended period of time.

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plasmapheresis in combination with a biotin affinity column. Through use of such column, circulating NR-LU avidin will be retained extracorporeally, and the recipient's immune

system exposure to a large, proteinaceous immunogen avidin) is minimized.

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useful in the practice of the present invention is discussed in the context of reducing radiolabeled antibody titer in imaging and in treating tumor target sites in U.S. Patent 5,078,673. Briefly, for the purposes of the present invention, an example of an extracorporeal clearance methodology may. . .

. . .

al., Biodegradable and Biocompatible Poly(DL-Lactide-Co-Glycolide) Microspheres as an Adjuvant for Staphylococcal Enterotoxin B Toxoid Which Enhances the Level of Toxin-Neutralizing Antibodies, Infection and Immunity, 59:2978-2986, 1991 (toxoid entrapment); Cohen et al., IfControlled Delivery Systems for Proteins Based on Poly(Lactic/Glycolic Acid) Microspheres, Pharmaceutical Research, 8(6):713-720, 1991 (enzyme entrapment); and Sanders. . .

. . .

involves administration of biotinylated, high molecular weight molecules, such as liposomes, IgM and other molecules that are size excluded from ready permeability to tumor sites. When such biotinylated, high molecular weight molecules aggregate with NR-LU avidin, the aggregated complexes are readily cleared from the circulation via. . .

. . .

from the circulation. One of ordinary skill in the art of radioimmunotherapy is readily able to determine the optimal time for hictinylated NR-CO-04 tumor localization and clearance from the circulation. At such time, the doxcrubicin-avidin conjugate is administered to the recipient. The avidin portion of the doxcrubicin-avidin conjugate. . .

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In a first alternative protocol, a standard three-step pretargeting methodology is used to enhance intracellular delivery of a drug to a tumor target cell.

Subsequent administration of avidin induces internalization of the complex and enhances intracellular delivery of drug to the tumor target cell.

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studies are useful to characterize the

reaction product, which studies include, for example, serum clearance profiles, ability of the conjugate to target antigen-positive tumors, tumor retention of the
conjugate over time and the ability of a biotinylated molecule to bind streptavidin conjugate at the tumor.

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the formation of a 1:1 streptavidin-NR-LU-10 3S whole antibody conjugate that exhibits blood clearance properties similar to native NR-LU-10 whole antibody, and 8 3
tumor uptake and retention properties at least equal to native NR-LU
For example, Figure 3 depicts the tumor uptake profile of the NR-LU streptavidin conjugate (LU StrAv) in comparison to a control profile of native NR-LU-10 whole antibody. LU. . .

. . .

2, 61 24f 72, 120 hours
after injection of radiolabeled bictinyl' molecule YR-LU st-re'otavidin has shown very consistent patterns cf blocd clearance and tumor uztake in the LS-180 animal model. A renrepentative profile is shown in Figure 4. When either PIP-ET or Re-BT is administered after allowing the LU StrAv conjugate to localize to target cell sites for at least 24 hours, the tumor uptake of theraneutic radionuclide is high in both absolute amount and rapidity. For PIP-ET administered at 37 hours following LU StrAv (I-125) administration, tumor uptake was above 500 pMOL/G at the 40 hour time point and peaked at about 700 pMOL/G at 4S hours post-LU StrAv administration.

This almost instantaneous untake of a small molecule therazeutic into tumor in stoichiometric amounts comparable to the antibody targeting moiety facilitates utilization of the therapeutic radionuclide at its highest specific activity. Also, the rapid clearance of radionuclide that is not bound to LU StrAv conjugate permits an increased targeting ratio (tumor:blood) by eliminating the slow tumor accretion phase observed with directly labeled antibody conjugates. The pattern of radionuclide tumor retention is that of whole antibody, which is very persistent.

Exam-ple XII7
Asialooroso=coid Clearing Agent and Two-Step Pretargeting
In order to maximize the targeting ratio (tumor:blocd), clearing agents were sought that are capable of clearing the blood pool of targeting moiety-anti-ligand conjugate (e.g., LU StrAv), without compromising the. . .

. . .

in Figure 9. Administration of 200 Ag biotinylated asialoorosomucoid resulted in a 50-fold reduction in serum biotin-binding capacity and, in preliminary studies in tumored animals, has not exhibited cross-linking and removal of prelocalized LU StrAv conjugate from the tumor.' Removal of circulating targeting moiety-anti-ligand without diminishing biotin-binding capacity at target cell sites, coupled with an increased radiation dose to the tumor resulting from an increase in the amount of targeting moiety-anti-ligand administered, results in both increased absolute rad dose

to tumor and diminished toxicity to non-tumor cells, compared to what is currently achievable using conventional radioimmunotherapy.

Examnle XIV

Tumor Uptake of PIP-Biocytin

PIP-Biocytin, as prepared and described in Example VII above, was tested to determine the fate thereof in vivo. The following data are based on experimentation with tumored nude mice (100 mg LS-180 tumor xenografts implanted subcutaneously 7 days prior to study) that received, at time 0, 200 Ag of I-125 labeled NR-LU Streptavidin conjugate (950. . . . of 42 Ag (69,767 pmol), 21 gg (34,884 pmol), 5.7 gg (9468 pmol), 2.85 jig (4734 pmol) or 0.5 jig (830 pmol). Tumors were excised and counted for radioactivity 4 hours after PIP-biocytin injection.

The three highest doses produced PIP-biocytin tumor localizations of about 600 pmol/g. Histology conducted on tissues receiving the two highest doses indicated that saturation of tumor-bound streptavidin was achieved.

Equivalent tumor localization observed at the 5.7 Ag dose is indicative of streptavidin saturation as well. In contrast, the two lowest doses produced lower abscluze tumor localization of PIP-biocytin, despite ecruivalelnt localization of NR-LU Streptavidin conjugate (tumors in all groups averaged about 400]o ID/G for the conjugate).

The lowest dose group (0.5]ig) exhibited high efficiency tumor delivery of PIP-I biocytin, which efficiency increased over time. A peak uptake of 85.0 i ID/G was observed at the 120 hour. . . (96 hours after administration of PIP-biocytin). Also, the absolute amount of PIP-biocytin, in terms of ']o ID, showed a continual increase in the tumor over all of the sampled time points. The decrease in uptake on a % ID/G basis at the 168 hour time point resulted from significant growth of the tumors between the 120 and 168 hour time points.

In addition, the co-localization of NR-LU Streptavidin conjugate (LU StrAv) and the subsequently administered PIP-Piocytin at the same tumors over time was examined. The localization of radioactivity at tumors by PIP-biocytin exhibited,a pattern of uptake and retention that differed from that of the antibody-streptavidin conjugate (LU StrAv). LU StrAv exhibited a characteristic tumor uptake pattern that is equivalent to historical studies of native NR-LU-10 antibody, reaching a peak value of 400i ID/G between 24 and 48 hours after administration. In contrast, the PIP-Biocytin exhibited an initial rapid accretion in the tumor, reaching levels greater than those of LU StrAv by 24 hours after PIP-Biocytin administration. Moreover, the localization of PIP-Biocytin continued to increase out. . .

The ratio of PIP-Diocytin to LU StrAv in the tumor increased continually during the experiment, while the ratio in the blood decreased continually. This observation is consistent with a process involving

continual binding of targeting moiety-containing conjugate (with PIP-13iocytin bound to it) from the blood to the tumor, with subsequent differential processing of the PIP-Diocytin and the conjugate. Since radiclabel associated with the streptavidin conjugate component (compared to radiolabel associated with. . . the targeting moiety) has shown increased retention in organs of metabolic processing, PIP-Biocytin associated with the streptavidin appears to be selectively retained by the tumor cells. Because radiolabel is retained at target cell sites, a greater accumulation of radioactivity at those sites results.

. . .
the absolute
AUCtumor for PIP-Biocytin is nearly twice that of the conjugate (9220 compared to 4629). Consequently, an increase in radiation dose to tumor was achieved.

Exam-ole XV
Clearing Agent Evaluation Experimentation
The following experiments conducted on non-tumor-bearing mice were conducted using female BALB/c mice (20-25 g). For tumor-bearing mice experimentation, female nude mice were injected subcutaneously with LS-180 tumor cells, and, after 7 d, the mice displayed 50-100 mg tumor xenografts. The monoclonal antibody used in these experiments was NR-LU When radiolabeled, the NR-LU-lo-streptavidin conjugate was radiolabeled with I-125 using procedures described herein.. . .

A. Utilitv of Asialoorosomuccid-Diotin (AO-Pt) in Reducincr Circulating Radioactivity from a Subse(:fuentlv Administered Radiolabeled Biotin Liaand. Mice bearing LS-180 colon tumor xenograftts were injected with 200 micrograms NR-LU-10 antibody-streptavidin (MAb-StrAv) conjugate at time 0, which was allowed to prelocalize to tumor for 22 hours. At. that time, 20 micrograms of AO-Et was administered to one group of animals. Two hours later, 90 micrograms. . . mice and also to a group which had not received
AO-Bt. The results of this experiment with respect to radiolabel uptake in tumor and clearance from the blood indicated that tumor-targeting of the radiolabeled biotin-containing conjugate was retained while blood clearance was enhanced, leading to an overall improvement in amount delivered to target/amount located.. . .

is The AUC tumor/AUC blood with clearing agent was 6.87, while AUC tumor/AUC blood without clearing agent was Blood clearance of the circulating MAb-StrAv conjugate was enhanced with the use of clearing agent.

The clearing agent was radiolabeled in a separate group of animals and found to bind directly to tumor at very low levels (1.7 pmol/g at a dose of 488 total pmoles (0.35%ID/g), indicating that it does not significantly compromise the ability of tumor-bound M.Ab-StrAv to bind subsequently administered radiolabeled ligand.

C. Dosina Ontimization of AO-Et. Tumored mice receiving StrAv-M.Ab as above, were injected with increasing doses of AO-Bt (O micrograms, 20 micrograms, 50 micrograms, 100 micrograms and 200 micrograms). Tumor uptake of I PIP-biocytin (5.7 micrograms, administered 2 hours after AO-Bt administration) was

examined. Increasing doses of AO-Bt had no effect on tumor localization of MAb-StrAv. Data obtained 44 hours after AO-St administration showed the same lack of effect. This data indicates that AO-Bt dose not cross-link and internalize MAb-StrAv on the tumor surface, as had been noted for avidin administered following biotinylated antibody.

PIP-biocytin tumor localization was inhibited at higher doses of AO-Et. This effect is most likely due to reprocessing and distribution to tumor of biotin used to derivatize AO-Bt. Optimal tumor to blood ratios ()O.

injected dose of radiolabeled ligand/gram weight of tumor is divided by 'i injected dose of radioligand/gram weight of blood were achieved at the 50 microgram dose of AO-Bt.

. . .

microgram AO-Bt dose revealed low retention of radiolabel in all non-target tissues (1.2 pmol/g in blood; 3.5 pmol/gram in tail; 1.0 pmol/g in lung; 2.2 pmol/g in liver; 1.0 pmol/g is spleen; 7.0 pmol/g in stomach; 2.7 pmol/g in kidney; and 7.7 pmol/g in intestine). With 99.3 pmol/g in tumor, these results indicate effective decoupling of the PIP-biocytin biodistribution from that of the MAb-StrAv at all sites except tumor. This decoupling occurred at all clearing agent doses in excess of 50 micrograms as well.

Decreases in tumor localization of PIP-biocytin was the significant result of administering clearing agent doses in excess of So micrograms. In addition, the amount of PIP-biocytin in non-target tissues 44 hours after administration was identical to localization resulting from administration of PIP-biocytin alone (except for tumor, where negligible accretion was seen when PIP-biocytin was administered alone), indicating effective decoupling.

D. Further Investigation of Optimal Clearing Agent Dcse. Tumored mice injected with MAb-StrAv at time 0 as above; 50 micrograms of AO-Bt at time 22 hours; and 545 microcuries of I. . .

Tumor uptake of 1 PIP-biocytin was preserved at the So microgram clearing agent dose, with AUC tumor/AUC blood of 30:1 which is approximately 15-fold better than the AUC tumor/AUC blood achieved in conventional antibody-radioisotope therapy using this model.

. . .

200 microgram dose of MAb-StrAv conjugate. The clearing agent administrations were followed 2 hours later by administration of 5.7 micrograms of I PIP-biocytin. Tumor uptake and blood retention of PIP-biocytin was examined 44 hours after administration thereof (46 hours after clearing agent administration). The results showed that. . . retention of PIP-biocytin was achieved by all doses greater than or equal to 40 micrograms of G-HSA-B. A clear, dose-dependent decrease in tumor binding of PIP-biocytin at each increasing dose of G-HSA-B was present, however. Since no dos.e-dependent effect on the localization of MAb-StrAv conjugate at the tumor was observed, this data was interpreted as being indicative of relatively higher blocking of tumor-associated MAb-StrAv conjugate by the release of biotin from catabolized

clearing agent. Similar results to those described earlier for the asialoorosomucoid clearing agent regarding plots of tumor/blood ratio were found with respect to G-HSA-B, in that an optimal balance between blood clearance and tumor retention occurred around the 40 microgram dose. Because of the relatively large molar amounts of biotin that could be released by this clearing. . . .

Comparison of these 9-, 5-, and 2-biotin-derivatized clearing agents with a single biotin G-HSA-B clearing agent was carried out in tumored mice, employing a 60 microgram dose of each clearing agent. This experiment showed each clearing agent to be substantially equally effective in blood clearance and tumor retention of MAb-StrAv conjugate 2 hours after clearing agent administration. The G-HSA-D with a single biotin was examined for the ability to reduce binding of a subsequently administered biotinylated small molecule (PIP-biocytin) in blood, while preserving tumor binding -biocytin to prelocalized MAb-StrAv conjugate.

of PIP
Measured at 44 hours following PIP-biocytin administration, tumor localization of both the KAb-StrAv conjugate and PIP-biocvtin was well preserved over a broad dose range of G-HSA-D with one hictin/molecule (90 9. . . 180 micrograms). A progressive decrease in blood retention of PIP-biocytin was achieved by increasing doses of the single biotin G-HSA-S clearing agent, while tumor localization remained essentially constant, indicating that this clearing agent, with a lower level of biotinylation, is preferred. This preference arises because the single. . .

Comparisons of tumor/blood localization of radiclabeled PIP-biocytin observed in the G-HSA-B dose ranging studies showed that optimal tumor to background targeting was achieved over a broad dose range (90 to 180 micrograms), with the results providing the expectation that even larger. . .

H. Tumor Targeting Evaluation Usina G-HSA-B. The protocol for this experiment was as follows.

Efficient delivery of the Lu DOTA-biotin small molecule was observed, 20-25 % injected dose/gram of tumor. These values are equivalent with the efficiency of the delivery of the MAb-StrAv conjugate. The AUC tumor/AUC blood obtained for this non-optimized clearing agent dose was 300% greater than that achievable by comparable direct MAb-radiolabel administration.

Subsequent experimentation has resulted in AUC tumor/AUC blood over 1000% greater than that achievable by comparable conventional MAb-radiolabel administration.
. . .
the administered therapeutic agent
conjugate as described supra, since it will further reduce the time the therapeutic agent is in contact with the immune system. Also, it is expected that these results can be extrapolated to other ligands or anti-ligands, as well as targeting moieties. . .

Examiple XX

Superantigen Pretargeting
A patient presents with colon cancer. A monoclonal antibody (MAb) directed to a colon cancer cell antigen, e.g., NR-LU-10, is conjugated to streptavidin to form a MAb-streptavidin conjugate. The MAb-streptavidin conjugate is administered to the patient in an. . .

The MAb-streptavidin so administered is permitted to localize to target cancer cells for 24 to 44 hours, preferably 48-72 hours.

. . .
sufficient to induce a cytotoxic effect at the target cell site). The biotinylated superantigen localizes to the targeted MAb- is streptavidin at the tumor or is metabolized or excreted from the patient via the renal pathway.

CLMEN. . . administering to the recipient a second conjugate comprising a highly toxic active agent selected from the group consisting of palytoxin, trichothecenes, Pseudcmonas exotoxin, tumor necrosis factor, interleukin-

40 The method of claim 38 wherein the targeting moiety is an annexin, an antibody which binds to activated platelets, or an anti-fibrin antibody.

45 The method of claim 44 when the cytokine is selected from interferons, interleukins, colony stimulating factors and tumor necrosis factors.

46 The method of claim 45 wherein the cytokine is a tumor necrosis factor.

=>

---Logging off of STN---

=>
Executing the logoff script...

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	27.47	27.68

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LOGINID:SSSPTA1642BJF

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* * * * * * * * * * Welcome to STN International * * * * * * * * * *

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